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ORIGINAL CLINICAL SCIENCE

Interleukin-17 receptor polymorphism predisposes to primary graft dysfunction after lung transplantation

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BACKGROUND: Primary graft dysfunction (PGD), with an incidence of 11% to 57%, is a major cause of morbidity and mortality within the first 30 days after lung transplantation (LTx). In this study, we postulate that recipient genetic variants in interleukin-17 and -23 receptor genes (IL-17R and IL-23R, respectively) may predispose LTx recipients to an increased risk for developing PGD.

METHODS: Seven genetic variants of IL-17R and IL-23R were successfully genotyped in 431 lung transplant recipients. Our primary end-point was PGD and secondary end-points were time to extubation, intensive care unit (ICU) stay, bronchoalveolar lavage neutrophilia and serum C-reactive protein.

RESULTS: The AA genotype of the rs882643 genetic variant of IL-17R was associated with higher PGD grades at 0 hour (adjusted $p = 0.042$), 12 hours (adjusted $p = 0.013$) and 48 hours (adjusted $p = 0.0092$) after LTx. The GG genotype of the rs2241049 genetic variant of IL-17R was associated with higher PGD grades at 48 hours (adjusted $p = 0.0067$) after LTx. For both genetic variants, no association was found with extubation time, ICU stay, post-operative BAL neutrophilia, serum CRP, chronic lung allograft dysfunction (CLAD) or graft loss.

CONCLUSION: Both genetic variants of IL-17R (rs882643 and rs2241049) were associated with PGD. This confirms a genetic predisposition toward PGD and suggests a role of IL-17 in driving neutrophilia in PGD.

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During the last 2 decades, lung transplantation (LTx) has become an established therapeutic option for selected patients with different forms of end-stage pulmonary disease. However, mortality rates after LTx remain relatively high.[1] Primary graft dysfunction (PGD), with an incidence of 11% to 57%, is a major cause of morbidity and mortality within the first 30 days after LTx.[1,2] PGD is characterized by pulmonary edema with diffuse alveolar damage, clinically manifesting as progressive hypoxemia with radiographic infiltrates.[2]

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PGD has been variously referred to over a wide range of synonyms, such as ischemia-reperfusion injury, re-implantation response, reperfusion edema, primary graft failure, post-transplant acute respiratory distress syndrome (ARDS) or acute lung injury (ALI). None of these terms are perfectly synonymous, and therefore in 2005 a working group of the International Society for Heart and Lung Transplantation (ISHLT) proposed a standardized definition for PGD, along with a grading system.[3] The defined criteria were radiographic pulmonary infiltrates and a partial pressure of oxygen/fraction of inspired oxygen (P/F) ratio assessed at certain time-points (0, 12, 24, 48, and 72 hours after LTx) with the exclusion of mechanical, immunologic and infectious causes that can mimic, modify or confound its definition and grading.[3] Injury inflicted on the donor lung by the transplant process (retrieval, preservation, implantation and reperfusion) in combination with other factors, such as acid aspiration, pneumonia and trauma from mechanical ventilation, contribute to the development of PGD.[2] This acute lung injury leads to alveolar flooding with protein-rich edema.[4] Furthermore, pulmonary injury will activate donor macrophages and pulmonary endothelial cells that recruit and activate recipient lymphocytes and neutrophils.[2] The inflammation leads to further epithelial damage and excessive cytokine secretion, which is probably the immunologic correlate of PGD and, ultimately, may even lead to graft loss.

The interleukin (IL)-17/IL-23 axis may play a key role in PGD, as demonstrated by Yoshida and colleagues, who showed that IL-17/IL-23-dependent memory T cells led to PGD-related bronchoalveolar lavage (BAL) neutrophilia.[5] Furthermore, genetic variants of IL-23R have been shown to be involved in chronic neutrophilic inflammatory diseases like rheumatoid arthritis, and Th17 cells are linked to several chronic inflammatory respiratory diseases, including chronic obstructive pulmonary disease, asthma, cystic fibrosis and bronchiolitis obliterans syndrome after LTx.[6–11] In the present study, we postulated that genetic variants in IL-17 receptor (IL-17R) and IL-23R genes may predispose LTx recipients to developing PGD after LTx.

Methods

Study design

In this retrospective analysis, we reviewed all LTxs performed at our hospital between January 2000 and December 2010. Approval for this study was granted by the institutional ethics committee of the University Hospitals Leuven (S54739) and all patients were asked to consent. The cohort consisted of 470 patients who received a single-lung (SLTx), sequential single-lung (SSLTx) or heart–lung (HLTx) transplant, and of whom clinical data, BAL fluid and blood were prospectively collected within our bio-bank (S51577).

Patients' characteristics or confounding factors included donor/recipient age, recipient gender, donor/receptor body mass index (BMI), bypass [cardiopulmonary bypass (CPB)/extracorporeal membrane oxygenation (ECMO)], donor P/F, ischemic time, surgery time, donor smoking history, sarcoidosis, type of LTx (SLTx vs SSLTx vs HLTx) and type of underlying lung disease. Clinical follow-up data included PGD, time to extubation (days), length of intensive care unit (ICU) stay (days), time to chronic lung allograft dysfunction (CLAD) and graft loss, BAL cell profile and serum C-reactive protein (CRP) as markers for pulmonary and systemic inflammation. PGD was graded at fixed time-points, 0, 12, 24 and 48 hours after LTx (T0, T12, T24 and T48), according to the ISHLT classification and based on P/F ratio (Grade 0 to 1: P/F >300 mm Hg; Grade 2: P/F 200 to 300 mm Hg; Grade 3: P/F <200 mm Hg). Post-operative (within the first week) bronchoscopies with BAL (2×50 ml of saline) in combination with cell count/differentiation were performed routinely, as previously reported.[12] The routine bio-banking of BAL at our center was started in 2001. Serum CRP values at 24, 48 and 72 hours were used for analysis. Retransplantation ($n = 18$) was considered as a separate transplantation for outcome analyses, because a second allograft was evaluated in an already genotyped recipient, as previously described.[13]

Genotyping

Of the total cohort of 470 lung transplant patients eligible for inclusion, recipient DNA was extracted from peripheral blood, or when unavailable from explanted lung tissue. DNA from blood samples was extracted using the QIAamp DNA Blood Midi kit, according to the supplier's instructions (Qiagen, Hilden, Germany). For DNA extraction from lung tissue embedded in paraffin blocks, paraffin was removed by xylene, followed by 2 washes with ethanol. After the paraffin-removal step, tissue was digested with proteinase K solution and DNA was extracted using a DNeasy Blood and Tissue kit (Qiagen). For control of DNA purity, 1 µl of genomic DNA was used to measure the 260/280 and 260/230 ratios with a Nanodrop 1000 (NanoDrop Technologies, Wilmington, DE). Only samples with a 260/280 ratio <2 and a 260/230 ratio <1 were accepted. DNA (5 ng/µl) was aliquoted into 384-well plates, and IL-17R and IL-23R genetic variants were genotyped in a blinded manner using iPLEX technology on a compact analyzer (MassARRAY; Sequenom, Inc., San Diego, CA), as reported previously.[7,11,14,15] In 16 patients, DNA extraction failed, and therefore no DNA was available for genotyping.

Genotyping for rs879574 (IL-17R gene), rs882643 (IL-17R gene), rs2241049 (IL-17R gene), rs2201841 (IL-23R gene), rs10489628 (IL-23R gene), rs2066808 (IL-23R gene) and rs134315 (IL-23R gene) was performed in a blinded manner using iPLEX technology on the compact analyzer (MassARRAY), as reported previously.[7,14,15] Automated genotyping calls were generated using the MassARRAY RTTM software and were validated by manual review of the raw mass spectra. Quality control was performed by genotyping 12 samples in duplicate, with a duplicate concordance of 100%. The success rate of genotyping within the remaining 454 patients was $>90\%$ (90% to 96%) for each of the 7 selected genetic variants.

Initially, an association between PGD at different time-points and any of the selected genetic variants was assessed. We could not find an association for any of the genetic variants in the IL-23R genes, but 2 genetic variants (rs882643 and rs2241049) of the IL-17R variant seemed to influence PGD after LTx and only those were investigated more closely. The success rate of genotyping within the remaining 454 patients was 95% for both single-nucleotide polymorphisms (SNPs; rs882643 and rs2241049), resulting in 431 patients for further analysis. The genetic variants are located in both coding (rs882643) and non-coding (rs2241049) regions of the IL-17R gene.

For rs882643, patients were sub-divided according to the risk genotype (AA). The AA genotype ($n = 33$) was analyzed versus AG/GG ($n = 398$). This sub-division is consistent with a recent report identifying an increased risk of Crohn's disease in patients carrying the rs882643 AA genotype.[16] For rs2241049, patients were sub-divided according to the risk genotype (GG). The GG genotype ($n = 62$) was analyzed versus AA/AG ($n = 369$).

Statistical analysis

For unadjusted statistical analysis of patient characteristics and outcome parameters, GraphPad PRISM 4.0 software (GraphPad, San Diego, CA) was used. Results are presented in numbers (percentage) or as median (± interquartile range). The Mann–Whitney *U*-test (non-parametric *t*-test) and chi-square test (contingency tables) were applied, where appropriate. The impact on outcome of time to CLAD and graft loss was analyzed by the Kaplan–Meier method. For further adjusted statistical analysis, SAS version 9.2 (SAS Institute, Inc., Cary, NC) was used. For continuous variables (extubation time and ICU stay) a general linear model was constructed, and for categorical variables (PGD) an ordinal logistic regression analysis was applied to estimate the *F*-value and odds ratio, respectively. For each outcome parameter, we performed a stepwise deletion model where we started adjusting for the following risk factors: donor age; donor BMI; donor P/F; date of LTx; type of LTx; bypass (cardiopulmonary bypass/extracorporeal membrane oxygenation); recipient age; recipient gender; recipient BMI; underlying disease; and sarcoidosis. A further sub-analysis was performed for the following risk factors: ischemia; surgery time; and donor smoking history. $p < 0.05$ was considered significant.

Results

Population characteristics

For rs882643, 33 patients (8%) carried the AA (genotype at risk), 154 (36%) the AG and 244 (56%) the GG genotype. Patients' characteristics did not differ for donor age ($p = 0.053$), recipient age ($p = 0.78$), recipient gender ($p = 0.88$), time of follow-up ($p = 0.057$), type of LTx ($p = 0.74$) or underlying lung disease ($p = 0.52$) (Table 1).

For rs2241049, 175 patients (41%) carried the AA, 194 (45%) the AG and 62 (14%) the GG (risk) genotype. Patients' characteristics did not differ for donor age ($p = 0.25$), recipient age ($p = 0.74$), recipient gender ($p = 0.80$), time of follow-up ($p = 0.74$), type of LTx ($p = 0.37$) and underlying lung disease ($p = 0.19$) (Table 1).

Table 1 Patients' Characteristics for rs882643 (IL-17R) and rs2241049 (IL-17R)

Characteristics	AA	AG/GG	*p*
rs882643			
Number of patients (*n*)	33	398	
Donor age (years)	36 (27–49)	45 (29–53)	0.053
Ischemic time (min)	382 (294–467)	367 (307–427)	
Recipient age (years)	52 (42–59)	53 (39–59)	0.78
Recipient gender (M) [*n* (%)]	18 (55)	211 (53)	0.88
Type of LTx (SL) [*n* (%)]	6 (18)	82 (21)	0.74
Time of follow-up (years)	6.1 (3.4–7.4)	4.6 (2.5–7.4)	0.057
Underlying lung disease pre-LTx			0.52
Emphysema, [*n* (%)]	13 (40)	188 (47)	
ILD [*n* (%)]	7 (21)	61 (15)	
Bronchiectasis/CF [*n* (%)]	6 (18)	71 (18)	
PPH [*n* (%)]	5 (15)	34 (9)	
Other [*n* (%)]	2 (6)	44 (11)	
Characteristics	AA/AG	GG	*p*
rs2241049			
Number of patients (*n*)	369	62	
Donor age (years)	44 (29–52)	48 (26–55)	0.25
Ischemic time (min)	367 (305–426)	382 (307–462)	
Recipient age (years)	53 (40–59)	53 (36–59)	0.74
Recipient gender (M) [*n* (%)]	197 (53)	32 (52)	0.80
Time of follow-up (years)	4.7 (2.6–7.5)	4.8 (2.7–6.8)	0.74
Type of LTx (SL) [*n* (%)]	78 (21)	10 (16)	0.37
Underlying lung disease pre-LTx			0.19
Emphysema, [*n* (%)]	174 (47)	26 (42)	
ILD [*n* (%)]	58 (16)	10 (16)	
Bronchiectasis/CF [*n* (%)]	69 (19)	8 (13)	
PPH [*n* (%)]	33 (9)	6 (10)	
Other [*n* (%)]	35 (9)	12 (19)	

Characteristics of the recipient cohort, sub-divided according to carrying the risk genotype. Results are shown as number (*n*) or with the median [IQR]. CF, cystic fibrosis; IL-17R, interleukin-17 receptor; ILD, interstitial lung disease; LTx, lung transplantation; M, male; PPH, primary pulmonary hypertension; SL, single-lung transplantation.

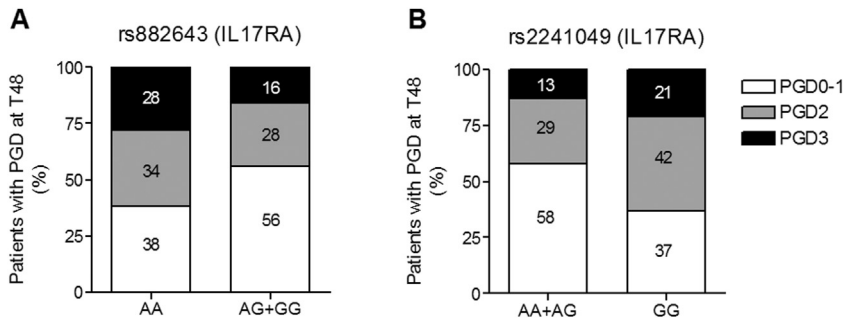

Figure 1 Patients carrying the risk genotype develop higher grades of PGD 48 hours after lung transplantation. (A) Patients with the AA genotype of the rs882643 (IL-17R) genetic variant more often developed Grade 3 PGD (28%) 48 hours after LTx compared with patients carrying the AG/GG genotype (16%). (B) Patients with the GG genotype of the rs882643 (IL-17R) genetic variant more often developed PGD Grade 3 (21%) and Grade 2 (42%) 48 hours after LTx compared with patients carrying the AA/AG genotype (13% and 29%, respectively). Results are shown as percentage (%). IL-17R, interleukin-17 receptor; PGD, primary graft dysfunction; T48, 48 hours after lung transplantation.

Associations between rs882643 (IL-17R) and early outcome parameters

We identified an association between rs882643 and PGD. More specifically, we observed an increase in PGD grades at T0 (unadjusted $p = 0.084$, adjusted $p = 0.042$), T12 (unadjusted $p = 0.0090$, adjusted $p = 0.013$) and T48 (unadjusted $p = 0.061$, adjusted $p = 0.0092$), which was seen more often in recipients carrying the rs882643 AA genotype, confirming that the AA genotype is the at-risk genotype for rs882643. Especially at T48, we observed that the patients with the AA genotype more often developed PGD Grade 3 (28%) compared with patients with the AG/GG genotype (16%) (Figure 1A). A trend was observed for PGD at T24 (unadjusted $p = 0.069$, adjusted $p = 0.074$), but no difference was seen in time to extubation ($p = 0.055$) and

Table 2 Unadjusted and Adjusted Analysis of Outcome Parameters for rs882643 (IL-17R, upper part) and rs2241049 (IL-17R, lower part)

			Unadjusted analysis	Adjusted analysis	
	AA	AG/GG	*p*	Odds ratio (CI)	*p*
rs882643 outcome parameters					
PGD T0 (Grade 0–1/2/3/NA, %)	12/21/55/12	32/18/44/6	0.084	0.44 (0.19–1.00)	0.051
PGD T12 (Grade 0–1/2/3/NA, %)	24/39/27/10	51/21/21/7	0.0090	0.48 (0.23–0.98)	0.043
PGD T24 (Grade 0–1/2/3/NA, %)	42/18/30/10	54/24/15/7	0.069	0.58 (0.29–1.18)	0.13
PGD T48 (Grade 0–1/2/3/NA, %)	36/30/24/10	53/28/12/7	0.079	0.45 (0.22–0.92)	**0.028**
Time to extubation (days)	5 (3–10)	4 (2–7)	0.055		0.94
ICU stay (days)	9 (5–16)	7 (5–13)	0.26		0.98
CLAD-free survival (years)	5.4 (2.8–6.7)	3.7 (1.8–6.2)	0.097	0.53 (0.25–1.14)	0.11
Graft loss (years)	6.1 (3.4–7.3)	4.6 (2.5–7.4)	0.49	0.77 (0.38–1.53)	0.45
	AA/AG	GG	*p*	Odds ratio (CI)	*p*
rs2241049 outcome parameters					
PGD T0 (Grade 0–1/2/3/NA, %)	111/66/168/24	19/11/27/5	0.98	0.98 (0.56–1.70)	0.95
PGD T12 (Grade 0–1/2/3/NA, %)	178/81/82/28	30/17/10/5	0.44	0.88 (0.51–1.54)	0.67
PGD T24 (Grade 0–1/2/3/NA, %)	194/85/62/28	32/16/9/5	0.84	1.06 (0.61–1.85)	0.83
PGD T48 (Grade 0–1/2/3/NA, %)	54/27/12/7	36/39/19/6	**0.017**	2.27 (1.33–3.88)	**0.0027**
Time to extubation time (days)	4 (2–7)	4 (3–10)	**0.039**		0.29
ICU stay (days)	7 (4–13)	9 (5–15)	**0.024**		0.23
CLAD-free survival	3.8 (1.9–6.4)	3.1 (1.7–5.7)	0.27	0.77 (0.48–1.23)	0.27
Graft loss	4.7 (2.6–7.5)	4.8 (2.7–6.8)	0.33	0.84 (0.54–1.30)	0.43

Results are presented as number (*n*) or median [IQR]. Adjusted analysis was performed applying a stepwise deletion based on a general linear model for categorical variables (PGD) and an ordinal logistic regression analysis for continuous variables (time to extubation and ICU stay). Bold *p*-values are statistically significant. For both genetic variants (rs882643 and rs2241049) adjusted analysis (stepwise deletion model) was performed while adjusting for these risk factors: PGD T0: donor age, donor P/F, date of LTx, recipient age, recipient gender and recipient BMI; PGD T12: donor age, donor P/F, recipient age and recipient BMI; PGD T24: date of LTx, type of LTx, bypass and recipient BMI; PGD T48: donor age, LTx type and recipient BMI; Time to extubation: donor age, underlying disease and bypass; ICU stay: underlying disease and bypass; CLAD-free survival: donor age, underlying disease, LTx type and sarcoidosis; Graft loss: donor age, recipient gender, recipient BMI, underlying disease, LTx type, donor BMI and donor P/F ratio. CI, confidence interval; ICU, intensive care unit; IL-17R, interleukin-17 receptor; PGD, primary graft dysfunction; T0, T12, T24 and T48: 0, 12, 24 and 48 hours after LTx.

ICU stay ($p = 0.26$) (Table 2, upper part). It would be of interest to further explore the association between the genetic variants and PGD at T72 after LTx. However, due to the large number of patients lacking data at this time-point ($n = 166$), we would lose statistical power. We did assess the stepwise deletion model for each genetic variant and PGD T72, but no significant difference was observed.

Associations between rs2241049 and early outcome parameters

We identified an association between rs2241049 and PGD. More specifically, increases in PGD Grade 2 (GG: 38.7%; AA/AG: 27.1%) and Grade 3 (GG 19.4%; AA/AG 12.2%) at T48 (unadjusted $p = 0.014$, adjusted $p = 0.0067$) were seen more often in recipients carrying the rs2241049 GG genotype, suggesting that the GG genotype is the at-risk genotype for rs2241049. No significance was observed for PGD at T0 (unadjusted $p = 0.98$, adjusted $p = 0.97$), T12 (unadjusted $p = 0.44$, adjusted $p = 0.73$) and T24 (unadjusted $p = 0.84$, adjusted $p = 0.93$). Especially at T48, patients with the GG genotype more often developed PGD Grade 3 (21%) and PGD Grade 2 (42%) compared with patients carrying the AG/GG genotype (13% and 29%, respectively) (Figure 1B). We found no difference for time to extubation (unadjusted $p = 0.039$, adjusted $p = 0.43$) and ICU stay (unadjusted $p = 0.024$, adjusted $p = 0.58$) (Table 2, lower part). It would be of interest to assess the effect of the combination of both at-risk genotypes on outcome parameters, but unfortunately none of the patients in our study cohort ($n = 431$) carried this combination.

Ischemia, surgery time and donor smoking history are major confounders. Because of the incomplete data regarding our study population, which would jeopardize the statistical power of our study, we did not include them in our stepwise deletion model. Therefore, we performed a sub-analysis while adjusting for these risk factors separately. This sub-analysis tends to show similar results to the data shown in this study.

Associations between rs882643 and rs2241049 with BAL neutrophilia and serum CRP

To explore the potential functional relevance of rs882643 and rs2241049 for early outcome after LTx, we assessed the association with BAL neutrophilia and serum CRP concentration measured after LTx. For rs882643, no difference was found in serum CRP levels between the AA genotype and the AG/GG genotype at 24 ($p = 0.47$; 82 [26 to 106] vs 91 [29 to 145] mg/liter), 48 ($p = 0.55$; 136 [72 to 187] vs 120 [86 to 159] mg/liter) or 72 ($p = 0.76$; 101 [60 to 155] vs 93 [63 to 135] mg/liter) hours after LTx (Figure 2A, C and E). No difference was observed in percentage of neutrophils in BAL ($p = 0.19$; 67% [39% to 85%] ($n = 24$) vs 51% [31% to 80%] ($n = 232$)) between the 2 groups (Figure 2G). For rs2241049, no difference was found in concentration of serum CRP between the GG genotype and the AA/AG genotype at 24 ($p = 0.91$; 90 [28 to 145] vs 89 [41 to 127] mg/liter), 48 ($p = 0.59$; 122 [87 to 159] vs 111 [72 to 169] mg/liter) or 72 ($p = 0.89$; 94 [63 to 137] vs 92 [59 to 134] mg/liter) hours after LTx (Figure 2B, D and F). No difference was observed in percentage of neutrophils in BAL ($p = 0.42$; 58% [36% to 78%] ($n = 224$) vs 54% [29% to 82%] ($n = 38$)) between the 2 groups. We were unable to obtain data from all patients included in the study because taking BAL samples has only been part of the standardized clinical care of LTx recipients since 2003.

Associations between rs882643 or rs2241049 and long-term outcome

For rs882643, the median time of follow-up was similar in patients with the risk genotype (AA) compared with patients with the other genotypes (AG/GG) ($p = 0.057$). Both CLAD-free survival (at 10 years: AA 60%; AG/GG 42%) (unadjusted $p = 0.097$, adjusted $p = 0.11$) and graft loss (at 10 years: AA 46%; AG/GG 50%) (unadjusted $p = 0.49$, adjusted $p = 0.45$) were similar between the 2 groups (Figure 3A and C, respectively). For rs2241049, the median time of follow-up was similar in patients with the risk genotype (GG) compared to patients with the other genotypes (AA/AG) ($p = 0.74$). Both CLAD-free survival (at 10 years: GG 44%, AG/AG 50%) (unadjusted $p = 0.27$, adjusted $p = 0.27$) and graft loss (at 10 years: GG 25%; AA/AG 47%) (unadjusted $p = 0.33$, adjusted $p = 0.43$) were similar between the 2 groups (Figure 3B and D, respectively).

Discussion

In this study we have identified the role of IL-17R genetic variants (rs882643 and rs2241049) in PGD after LTx. Patients carrying the risk genotypes (AA or GG, respectively) had an increased risk to develop higher PGD grades, in particular at T48 after LTx, compared with patients carrying the AG/GG ($p = 0.0092$) and AA/AG ($p = 0.0067$) genotype, respectively. For both genetic variants we could not find a difference in time to extubation, ICU stay, serum CRP or BAL neutrophilia.

The loss of the integrity of the alveolar-capillary barrier is a key element in developing PGD after LTx.[4] Under physiologic conditions, the epithelial barrier is much less permeable compared with the endothelial barrier.[17] Acute lung injury after LTx disrupts the epithelial integrity and interferes with both Type I and Type II epithelial cell function, which contributes to the onset of PGD.[17,18] Sloughing of Type I epithelial cells with denudation of the basement membrane in combination with endothelial injury causes influx of protein-rich edema fluid into the alveolar space. Simultaneously, neutrophils adhere to the injured endothelium, which marginate to the interstitium and eventually migrate into the alveolar space. Alveolar macrophages secrete cytokines (e.g., IL-1, -6, -8 and -10, and tumor necrosis factor-α), which in turn stimulate chemotaxis and activation of neutrophils.[4] Activation of these neutrophils within the alveolar space results in the release of oxidants, proteases, leukotrienes and other pro-inflammatory molecules, which cause further damage to the Type II

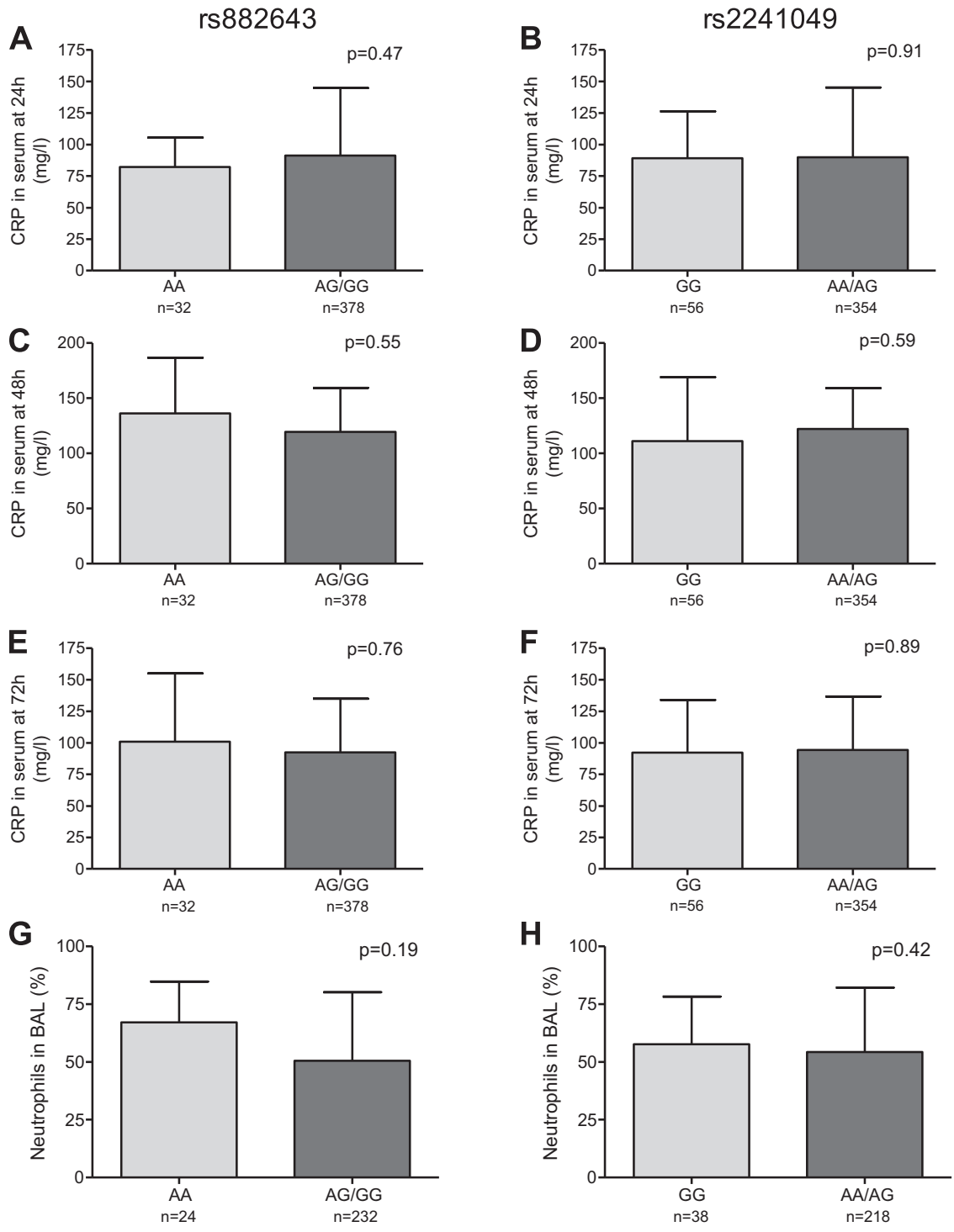

Figure 2 CRP concentration in serum and neutrophil percentage in BAL. (A, C, E) CRP concentration measured 24 ($p = 0.47$), 48 ($p = 0.55$) and 72 ($p = 0.76$) hours after LTx did not differ between patients with the AA genotype and those with the AG/GG genotype in the rs882643 variant. (B, D, F) CRP concentrations measured 24 ($p = 0.91$), 48 ($p = 0.59$) and 72 ($p = 0.89$) hours after LTx did not differ between patients with the GG genotype and those with the AA/AG genotype in the rs2241049 variant. The percentage of neutrophils measured in the post-operative BAL sample did not differ between groups for rs882643 ($p = 0.19$) (G) and rs2241049 ($p = 0.42$) (H) genetic variants. Results are shown as median [IQR]. CRP, C-reactive protein; BAL, bronchoalveolar lavage.

epithelial cells. Injury to Type II epithelial cells interferes with normal epithelial fluid transport impairing the removal of edema fluid from the alveolar space.[19] Type II epithelial damage and alveolar flooding reduces the production and turnover of surfactant, further impairing removal of edema fluid. The characteristic abnormalities in production, composition and function of surfactant also result in alveolar collapse and gas-exchange impairments.[20] A last mechanistic element playing a role in development of PGD may be aberrant coagulation, which could lead to platelet-fibrin thrombi formation in small vessels and impaired fibrinolysis.[18,21]

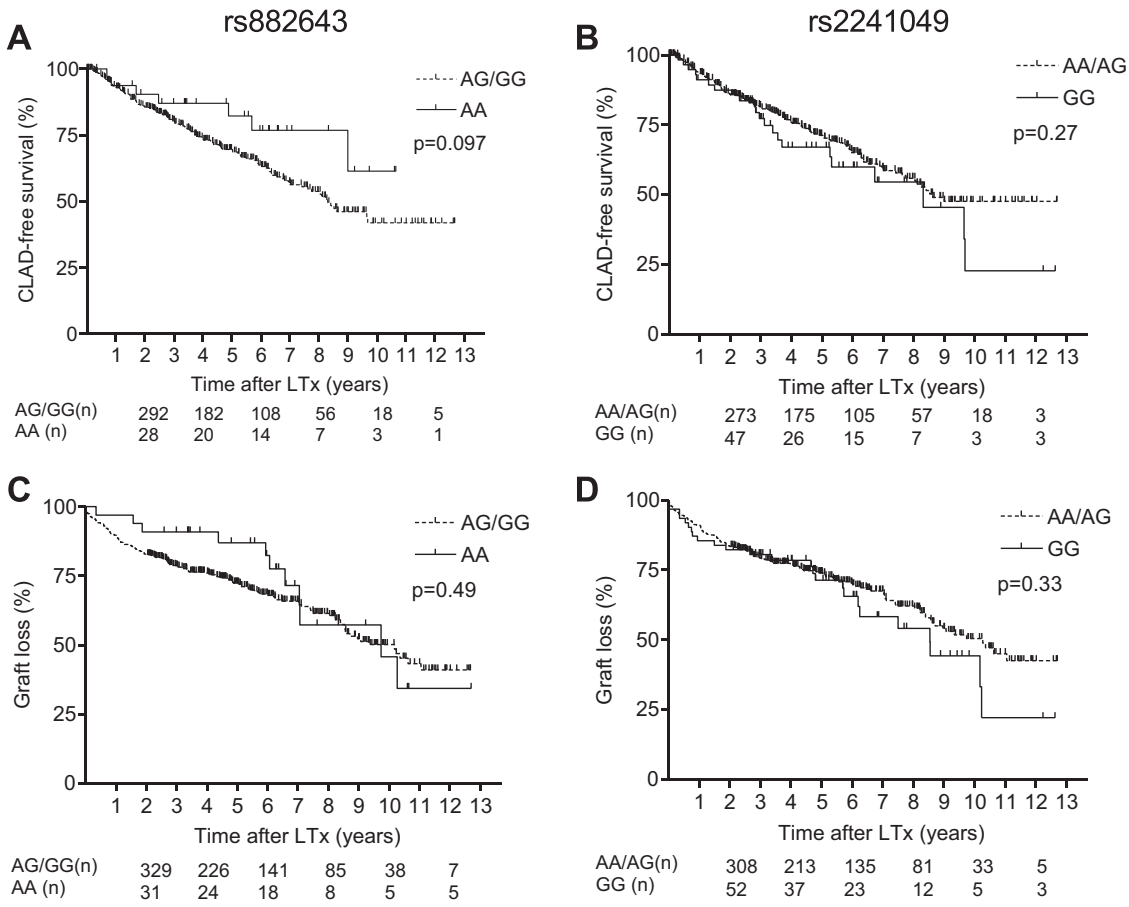

Figure 3 CLAD-free survival and graft loss. (A, C) There was no difference in CLAD-free survival or graft loss for patients with the AA genotype of the rs882643 (IL-17R) genetic variant. (B, D) There was no difference in CLAD-free survival or graft loss for patients with the AA genotype of the rs2241049 (IL-17R) genetic variant.

The onset of PGD has many triggers including aspiration, prolonged ischemic time, reperfusion injury, microbial involvement and immune mismatch.[2] Only 1 risk factor, genetic predisposition, was not investigated, until the study by Diamond and colleagues.[22,23] They demonstrated the involvement of genetic variants of genes encoding proteins (PTX3) and hormones (PGE_2) in the pathogenesis of PGD.[22,23] In our study of 431 patients, the neutrophil axis was targeted by investigating the major inducer of neutrophilia, IL-17. The observed association between PGD and a SNP in the IL-17R confirms the involvement of this pathway in PGD through a genetic predisposition. Currently, production of IL-17 is considered to be restricted to a number lymphocytes of both adaptive subsets, such as T-helper 17 (Th17), T-cytotoxic 17 (Tc17), and innate subsets, such as T-follicular helper 17 (Tfh17), natural killer T 17 cells (NKT-17) and gamma delta T 17 (γδT17).[24] Unlike IL-17 itself, IL-17RA is expressed ubiquitously on structural and immune cells but can be dynamically regulated by, for instance, IL-15, IL-21 and phosphoinositide 3-kinase (PI3K).[25] The IL-17R family comprises the 5-subunit IL-17RA–IL-17RE, of which IL-17RA is by far the largest member.

Although IL-17RA is the most needed for IL-17A binding, optimal IL-17A binding is only accomplished by formation of a productive receptor complex together with IL-17RC.[26] The IL-17RA polymorphisms assessed were located on an intron (between exon 12 and 13) (rs2241049) and on exon 13 (rs882643). The SNP located in the intron has no influence on the protein structure. On the other hand, the mRNA of IL-17RA consists of an unusually short 5′UTR region followed by the start codon, the protein coding region, and finally the stop codon followed by an unusually long 3′UTR region. This rs882643 SNP variant located on exon 13 is part of the 3′UTR regions and will not cause a change in amino acid, but it will affect the stability of the mRNA. However, we do not know on which of the 5 functional regions of IL-17RA, 2 extracellular fibronectin III-like domains, a transmembrane domain or the intracellular SEFIR (similar expression to fibroblast growth factor genes and IL-17R), TILL (Toll/IL-1R-like loop) or CBAD (CCAAT/enhancer binding proteins β-activation domain) domains, it will have an impact.

The presence of the risk genotype in recipients will most probably lead to a gain of function with an increase in IL-17Rα on structural cells and immune cells at time of transplantation. Within the recipient, it will lead to enhanced proliferation and differentiation of IL-17 T cells as IL-17 itself stimulates immune cell activation (direct positive feedback loop) via IL-17Rα and, in addition, structural cells will increase IL-17Rα production, which will lead to increased activation of IL-17-producing cells releasing

IL-17, cell activation and differentiation cytokines and growth factors such as IL-1β, IL-6, IL-23 and transforming growth factor-β (indirect feedback loop). An increased presence of these cells immediately after transplantation within the recipient will cause an increased number of infiltrating cells in the donor lung allograft at time of transplantation and will facilitate in onset of PGD.

The absence of a link between the IL-17R SNP and BAL neutrophilia remains a shortcoming. Yet, this may be explained by the lack of paired BAL samples at this time-point after LTx ($n = 256$ for rs882643 and $n = 262$ for rs2241049). Furthermore, not all BAL samples were collected on the same post-operative day (variation between 1 and 7 days post-LTx) due to technical/logistic difficulties. Our single-center approach has its pros and cons but it leads to a more standardized and uniform care, which reduces possible bias by procedure and therapeutic regimen.

PGD is an acute event that occurs within hours or days after LTx. Therefore, cells of the adaptive immune system are most likely not involved and IL-17-producing cells involved in PGD are probably part of the innate subset of the IL-17-producing T cells. This may explain the lack of involvement of IL-23R genes, which are not required for activation of innate IL-17-producing cells.

Our study and the genetic studies by Diamond and colleagues show that genetic background should be considered for PGD. Further, these studies indicate that some patients, with specific genetic background, may be more vulnerable in developing PGD compared to others. However, our study does not imply that we have to start genetic screening for this specific polymorphism in all patients. Since this was beyond the scope of our study and would be an overinterpretation of our results. It needs to be considered that this study only addresses 2 SNPs of the many present on the IL-17RA gene. Therefore polymorphism studies should play a major role in further PGD research. Finally, we can conclude that the direct impact of this study involves acquiring new insights in the role of IL17/IL-17RA in the mechanism of PGD, which could lead to treatments that block this pathway and resolve PGD. Currently, in Leuven, a placebo-controlled study administering azithromycin to recipients before LTx is recruiting patients with the ultimate goal of reducing PGD (NCT01915082).

In conclusion, we can say that we have identified a role of polymorphisms in the IL-17R gene for the risk of developing PGD after LTx. This confirms the involvement of the IL-17 T cell axis in PGD, making it a target for therapy.

Disclosure statement

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